AD_____

Award Number:  DAMD17-03-1-0047


TITLE:  Therapy Selection by Gene Profiling



PRINCIPAL INVESTIGATOR:  Simon W. Hayward, Ph.D.



CONTRACTING ORGANIZATION:  The Vanderbilt University
                                            Nashville, TN  37232-2103


REPORT DATE:  May 2008


TYPE OF REPORT:  Final


PREPARED FOR:  U.S. Army Medical Research and Materiel Command
                        Fort Detrick, Maryland  21702-5012


DISTRIBUTION STATEMENT: Approved for Public Release;
                                         Distribution Unlimited


The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

# REPORT DOCUMENTATION PAGE

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

| 1. REPORT DATE *(DD-MM-YYYY)* | 2. REPORT TYPE | 3. DATES COVERED *(From - To)* |
|---|---|---|
| 01-05-2008 | Final | 1 Apr 2003 – 30 Apr 2008 |

| 4. TITLE AND SUBTITLE | 5a. CONTRACT NUMBER |
|---|---|
| Therapy Selection by Gene Profiling | |
| | 5b. GRANT NUMBER |
| | DAMD17-03-1-0047 |
| | 5c. PROGRAM ELEMENT NUMBER |

| 6. AUTHOR(S) | 5d. PROJECT NUMBER |
|---|---|
| Simon W. Hayward, Ph.D. | |
| | 5e. TASK NUMBER |
| E-Mail: simon.hayward@vanderbilt.edu | 5f. WORK UNIT NUMBER |

| 7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) | 8. PERFORMING ORGANIZATION REPORT NUMBER |
|---|---|
| The Vanderbilt University<br>Nashville, TN 37232-2103 | |

| 9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) | 10. SPONSOR/MONITOR'S ACRONYM(S) |
|---|---|
| U.S. Army Medical Research and Materiel Command<br>Fort Detrick, Maryland 21702-5012 | |
| | 11. SPONSOR/MONITOR'S REPORT NUMBER(S) |

**12. DISTRIBUTION / AVAILABILITY STATEMENT**
Approved for Public Release; Distribution Unlimited

**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT**
The long term goal of this work is to develop a new prognostic tool with which to determine the response of a patient to a given therapy, with the view of providing the most appropriate treatments tailored to individual patients. The central hypothesis of this proposal is that a subset of the genes expressed in a prostate tumor can be used to predict response to specific therapeutic regimens. The purpose of this work is to generate predictive methods which will allow patients to be selected for specific treatment protocols. This report summarizes the progress achieved towards the specific aims, the problems encountered, data sets generated and lessons learned from the work undertaken as a part of this grant.

**15. SUBJECT TERMS**
Taxotere, Genomics, Pharmacogenomics, microarrays

| 16. SECURITY CLASSIFICATION OF: | | | 17. LIMITATION OF ABSTRACT | 18. NUMBER OF PAGES | 19a. NAME OF RESPONSIBLE PERSON<br>USAMRMC |
|---|---|---|---|---|---|
| a. REPORT | b. ABSTRACT | c. THIS PAGE | | | |
| U | U | U | UU | 12 | 19b. TELEPHONE NUMBER *(include area code)* |

**Table of Contents**

Final Report

PCRP Idea Development Award

DAMD 17-03-1-0047

Therapy selection by gene profiling

**P.I. Simon W. Hayward, PhD**


**Introduction**

In addition to describing activities in the last year of funding, this final report will summarize activities, problems and achievements over the duration of this grant. As stated from the outset the **long-term goal** of this work was to develop a new prognostic tool with which to determine the response of a patient to a given therapy, with the view of providing the most appropriate treatments tailored to individual patients. The **central hypothesis** being that a subset of the genes expressed in a prostate tumor can be used to predict response to specific therapeutic regimens. A subordinate aim was to catalogue genes that are regulated in response to treatment with Taxotere, in both responding and non-responding human prostate cancer tissue samples, since these genes might suggest additional targets for therapeutic intervention. **Note on this amended report.** The submitted final report did not include significant data from the final year of work since most of the findings were negative. In response to the reviewer's comments this amended version includes tables and graphs summarizing relevant data. We have not included the full microarray data set since this comprises several hundred thousand individual data points and would not be of use either to the reviewer or the general public if submitted here. This data set will however be submitted to an appropriate public database when a final publication describing this work is completed. We will inform DOD-PCRP of the final disposition of these data and the associated publication.


**Specific Aims, per original proposal**


**Specific aim 1. Gene expression in patient tissues.**

**Specific aim 2. Effects of Taxotere on xenografted human prostate cancer tissues.**

**Specific Aim 3. Data analysis and prognostic tool design.**

**Specific aim 4. Prediction of response of patients in a clinical trial of Taxotere based upon analysis of archived snap frozen tissue.**


This proposal was recognized from its inception as high risk but potentially high gain. The essential

model system which was proposed was to utilize a xenografting model developed by the P.I. as a platform for assessing the regulation of genes by Taxotere in susceptible versus non-susceptible tumors.

**Problems Encountered**

The funding of this proposal coincided with two changes in clinical practice which impacted our collection of tissues. The first was a general migration towards downstaging and grading of prostate tumors. This effect has been going on for some time, was recognized at the time of the proposal submission, and while adding some time to the sample collection provided no major problems. The issue is that, with the widespread use of PSA testing, the size of prostate tumors which are detected and the stage and grade of the disease generally seen clinically is slowly decreasing. Despite debatable issues of over treatment it would generally be argued that this is good news for patients. However, perversely, it is not positive news for researchers since the pool of tissue available for research is reduced. However as noted, while we did see a reduction in the number of samples from which the Tissue Acquisition Core could provide samples, this caused delays but was not otherwise a serious problem. This was documented in early annual reports.

The second clinical issue, the introduction of robotic laparoscopic surgery as the preferred means of prostatectomy at this institution (and many others), has however turned out to be much more problematic. While the impact on clinical outcomes of this robotic surgery are unclear, heavy marketing to the patient population has resulted in high demand for the procedure. So early samples used for this project, as with historical samples on which our preliminary data were based, were from prostates which had been removed by open prostatectomy. As time went on, however, the proportion of samples derived by the laparoscopic procedure rapidly increased. Our initial characterization of responses to Taxotere and the times needed to achieve these were performed using tissue from open prostatectomy samples.

We noted in previous annual reports that the quality of tissue derived from prostates which were resected by this technique was not as good as that seen in open prostatectomy samples. However we modified our procedures to allow recovery of control tissue in vivo. What was not clear at that time, but has come into focus in the last year is that the loss of blood supply seen by the prostatic tissue during laparoscopic surgery resulted in a preferential killing of tumor as compared to normal cels. Thus while the normal cells were able to recover reasonably well when grafted into SCID mouse hosts this was not true of the cancer tissues. As a result there was a significant reduction in the proportion of cancer tissues

seen in the samples derived from patients who underwent laparoscopic surgery.

**Activities in Final Year**

As described in the previous annual report we saw very low numbers of samples with evidence of apoptosis following Taxotere treatment when the tissue samples were collected and stained (examples shown in figure 1). This was in contrast to our preliminary data and to optimization experiments which were performed early in the study (importantly these studies used samples collected from open prostatectomy). It was important, for comparison purposes to perform all of the apoptosis staining at the same time, therefore the first comparisons could not be made until after the final tissues were harvested.
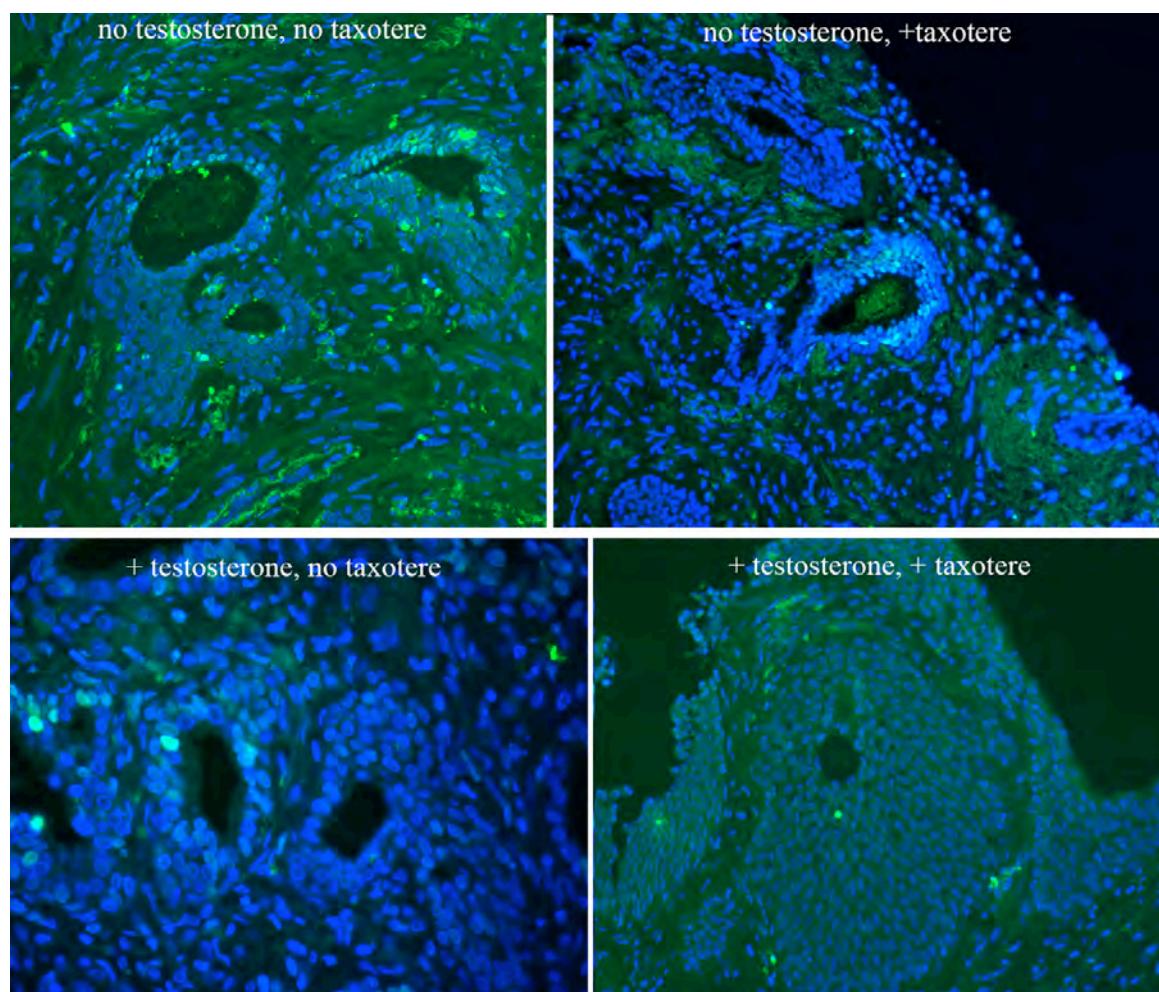


Figure 1. Examples of TUNEL (green) and DAPI (blue) staining of grafted human prostate tissue samples in mice treated as noted on the figures. The robust Taxotere response in well defined cancer tissue seen in preliminary studies was absent in the vast bulk of the tissue sections examined. Of note there was a marked absence of tumor tissue in most of the samples (quantitated in table 2).

The goals for the final year of funding were to ascertain the best method of assessing response to Taxotere, given the poor response seen when apoptosis was assessed. Once this was achieved we planned to hire a data manager to select from banked tissue samples for the work proposed in the final specific aim. We therefore optimized immunohistochemical staining protocols for the expression of a series of markers with the potential to identify cells responding to Taxotere. The chosen markers were:

Thymidine phosphorylase (figure 2), which is induced in a number of tumor types following Taxotere treatment, typically 5-10 fold. Increased levels are long-lasting (peak levels >10 days). This protein is controversial as a predictor for Taxotere response in a number of cancers, but was expected to be useful to confirm exposure of xenografts to therapeutic levels of Taxotere by comparison of treated and non-treated patient matched samples.
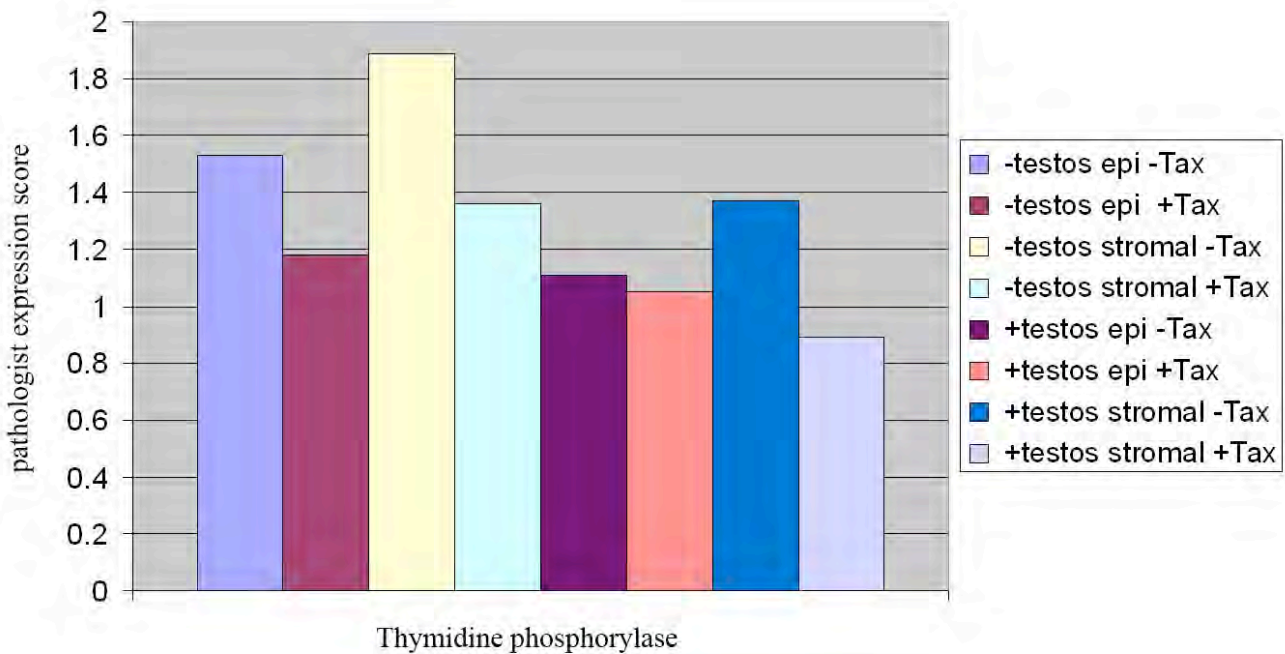


Figure 2. Relative thymidine phosphorylase staining intensity in stromal and epithelial compartments of grafted tissue samples with and without both testosterone and Taxotere, averages of samples. No trend was seen between enzyme staining and treatment protocol (shown here) and furthermore no consistent observations were made between patient matched treated and untreated pairs of samples (not shown) therefore no conclusions could be drawn in regard to Taxotere response.

Stabilized detyrosinated (Glu) microtubules, which are increased with Taxotere treatment. It was anticipated that this marker could be used in conjunction with thymidine phosphorylase levels to

confirm exposure of tumor xenografts to therapeutic levels of Taxotere, and might also be useful for detection of resistant tumor cells, as resistant cells often have beta-tubulin mutations that prevent microtubule stabilization.

Tubulin stabilization, which is a definitive positive response to Taxotere was also examined to determine which tumors contained cells in which microtubule structure has been locked, as a consequence of successful Taxotere treatment.

Thioredoxin, glutathione-S-transferase pi 1, and peroxidoxin 1 levels were also determined from microarray data, as these have been found in breast tumor biopsy samples to correlate with resistance to Taxotere response in patients (table 1 and figure 3).

| | | Signal value of spot for each tumor shown down the left side | | | | | |
|---|---|---|---|---|---|---|---|
| | | GSTP1 | INDO | PRDX1 | GSTP1 | TXN | TDO2 |
| | | vh034265 | vh025497 | vh024068 | vh024018 | vh021484 | vh019191 |
| Response ()*S | VMSR ID ()* | vh034265 | vh025497 | vh024068 | vh024018 | vh021484 | vh019191 |
| No apoptosis | 386SWH78 | 71 | 31748 | 42705 | 3771 | 27890 | 247 |
| No apoptosis | 386SWH90 | 245 | 783 | 43127 | 5776 | 53000 | 316 |
| No apoptosis | 386SWH91 | 212 | 1353 | 37183 | 3676 | 33233 | 416 |
| No apoptosis | 386SWH92 | 37 | 177 | 26407 | 595 | 28881 | 391 |
| No apoptosis | 386SWH93 | 172 | 756 | 30134 | 6075 | 30519 | 172 |
| Apoptosis | 386SWH74 | 94 | 3591 | 51485 | 6262 | 42442 | 173 |
| Apoptosis | 386SWH75 | 328 | 64998 | 60785 | 5167 | 40092 | 470 |
| Apoptosis | 386SWH84 | 213 | 7110 | 33633 | 7204 | 36157 | 265 |
| Apoptosis | 386SWH87 | 52 | 192 | 62530 | 2641 | 64678 | 1228 |
| Apoptosis | 386SWH89 | 85 | 2774 | 19023 | 2966 | 23038 | 85 |

Table 1. Microarray results comparing Taxotere treated samples with elevated apoptotic activity and those whose activity was not elevated. The purpose of this comparison was to determine whether this "response" seen in a small number of samples, was supported by changes in other potential markers of Taxotere action. The conclusion from the data presented here is that there was no correlation, suggesting, as we suspected, that the apoptotic response in these samples was not a reliable marker of Taxotere action. These data are shown graphically in pooled form in figure 3.
GSTP1=glutathione-S-transferase pi 1, INDO=indoleamine-pyrrole 2,3 dioxygenase ,PRDX1=peroxidoxin 1 ,TXN=thioredoxin, and TDO2=tryptophan 2,3-dioxygenase .
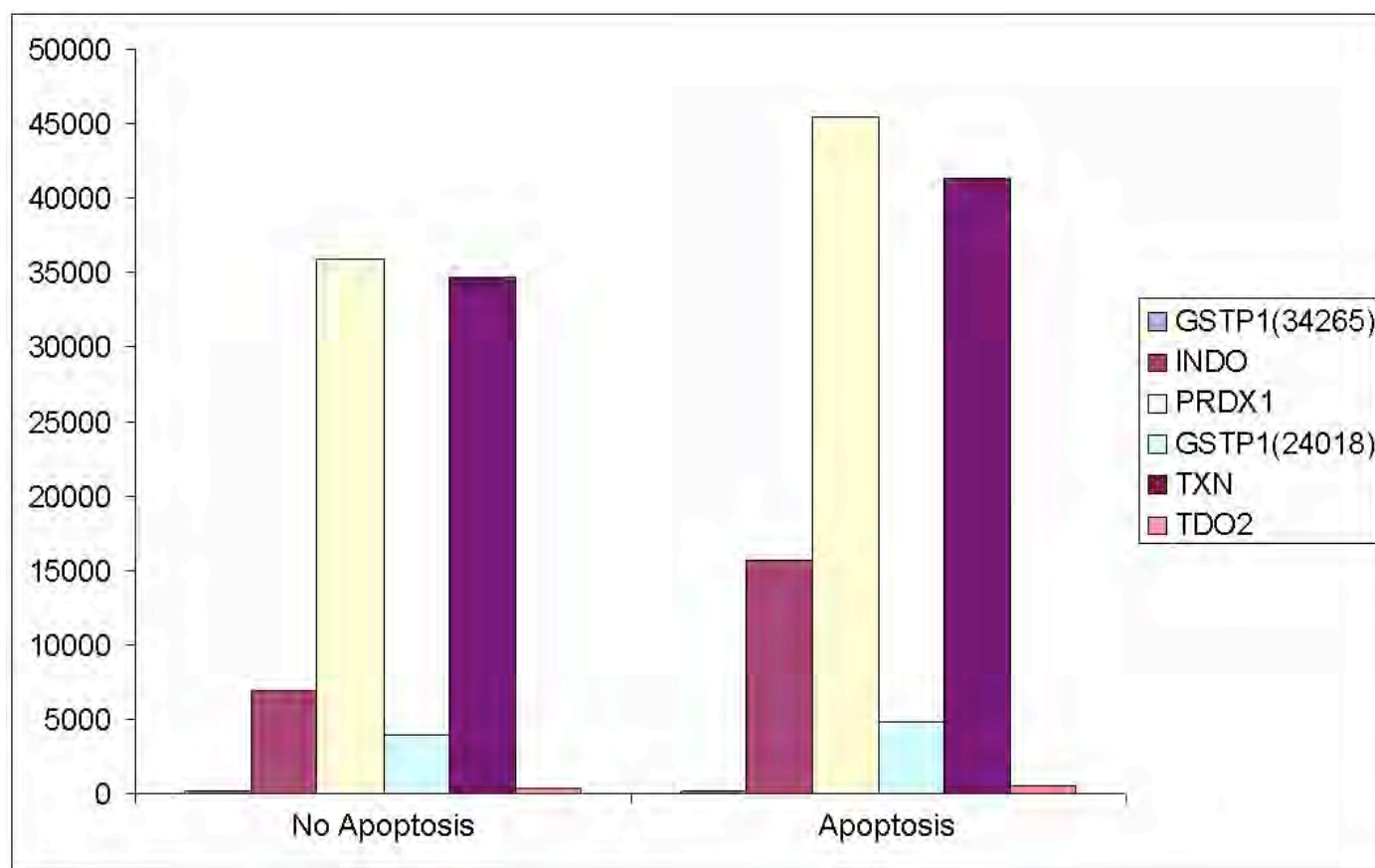
Figure 3. Graphical representation of the pooled microarray data from table 1. While the data from individual samples showed no clear trends some possible hints of overall response can be seen in relation to increases in PRDX1, INDO and TDO2 in the pooled data. However, as noted this analysis was, of necessity, restricted to a small sample set and therefore authoritative conclusions cannot be drawn.

Once the staining protocols were optimized slides were appropriately stained and examined critically in collaboration with a pathologist. This revealed that the lack of response seen in apoptosis was mirrored in these additional markers, however more importantly it also showed that there was little or no tumor tissue in many of the recovered samples, providing a reason for the lack of response (table 2). Given that our methods of assessing tumor going into the mice was constant (assessment of frozen sections immediately adjacent to the grafted samples) we did not think that the histopathology of the grafted tissue was likely to have changed. This loss of tumor tissue in the rescued fragments correlated with the switch to laparoscopic surgery, suggesting that the poor condition of the tissue presented after this procedure (as noted in earlier reports) was a much more significant problem than we had previously supposed. Our observation that tissues recovered to a condition that allowed the generation of good RNA samples did not take into account the nature of the cells from which such samples were derived.

| # of xenograft samples examined | 132 |
|---|---|
| # negative for malignancy | 111 |
| # with confirmed carcinoma | 11 |

Table 2. Summary of PCa xenograft pathology. Examination of samples from 132 evaluable xenografts showed that tumor was confirmed in only 8.3% of samples. As noted above this is in contrast to our preliminary studies, and also our published work in this area, where tumor in grafted samples was represented at almost 100% reliability. This change reflects alterations in clinical practice resulting in less well preserved tissue samples, as described in the text.

Given that we were unable to identify sufficient tumor tissue to complete the proposed studies a decision was made to terminate this aspect of the study and to return to DOD-PCRP the funds which had been earmarked for the hiring of a data manager to identify samples for specific aim 4 which we are therefore unable to complete.

**Original Statement of Work and Summary of Progress**

Task 1

Generate deoxyribose nucleic acid (DNA) Microarray patterns for prostate cancer samples from 150 patient tumor samples

a) As cases present, collect 150 histopathologically-confirmed prostate cancer tissue cores. Snap freeze core fragments (months 1-24) **Modified to allow recovery of tissues in vivo following switch to laparoscopic surgery. Completed.**

b) Prepare ribose nucleic acid (RNA) from snap frozen core fragments (months 1-25) **Completed.**

c) Run 150 comparative DNA microarrays using 12k human chip against a mixed sample human prostate standard (months 1-25). **Modified to use 30k oligonucleotide chips. Completed.**

Task 2.

*In vivo* studies

a) Perform preliminary study to determine optimal post-treatment time point for determining histopathological response to Taxotere (months 1-3). **Completed.**

b) Graft tissues from the cores used in task 1 to pairs of severe combined immune-deficient (SCID) mice (months 1-24). **Completed.**

c) After 30 days treat one of each pair of mice with Taxotere for 6 days (months 1-25). **Completed.**

d) Sacrifice mice and harvest tissues. Snap freeze tissues, make RNA for microarray analysis, take representative tissue samples for histology (months 2-26). **Completed.**

e) Run 300 comparative microarrays of untreated vs standard and Taxotere-treated vs standard samples (months 3-28). **Completed.**

Task 3

Biostatistical analysis

a) Identification of gene expression patterns which predict histopathologic response to Taxotere. Biostatistical analysis to determine a pattern of gene expression in tissue cores which predict histopathologic response to Taxotere in a xenograft model (months 26-32). **Not completed due to inability to identify responsive patients.**

b) Identification of genes regulated by Taxotere in responsive and non-responsive tissues. Biostatistical and bioinformatic analysis will be used to identify genes regulated by Taxotere in responsive and non-responsive tissue samples (months 28-34). **Analysis completed, data being prepared for publication.**

c) Design of assays/microarrays to predict response to Taxotere. Custom microarrays or assays (depending upon the number of prognostic genes identified in 3a) will be designed in which expression patterns of a limited number of genes should predict the response of human prostate cancer to Taxotere (months 32-35). **Not completed due to inability to identify responsive patients.**

Task 4

Prediction of response of patients in a clinical trial setting (months 35-36) **Not completed due to inability to identify sufficient numbers of responsive patients.**

**Technical modifications:**

No significant changes have occurred in the final budget period. Modifications were noted in previous reports as they occurred.

**Personnel Changes**

None

**Key Research Accomplishments**

• Samples collected and processed through animals.

- RNA prepared and amplified.
- Microarray analysis completed.
- Response outcomes determined.
- Biostatistical analysis of gene changes induced by Taxotere on human prostate tissues in vivo completed.

**Reportable Outcomes.**

None

**Conclusions.**

On a positive note this project has generated a large data set showing a profile of genes which are altered in human prostate tissues in vivo following challenge with Taxotere. We believe that these data should be in the public sphere and for this reason we are preparing a paper describing the experiments performed and the results obtained. This will make the full microarray data set available publicly for metaanalysis and for data mining by other interested groups.

The failure to achieve the main aim of the project is a great disappointment, especially since this was apparently due to changes in clinical practices which were well beyond our control, but which have implications for many other studies using human prostate tissues.

This work was always perceived as high risk but potentially very high gain. Clearly the failure to achieve the high gain is not to our satisfaction. It is particularly frustrating that this apparently results from a change in clinical practice which was beyond our control, which does not have established benefits to patients and whose adoption was largely driven by direct marketing to patients.